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PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Applicant:	Gregory Lee Durst	Group Art Unit: 1609
Serial No.:	10/552,504	Examiner: J. Mabry
International Application Date:	April 8, 2004	Conf No.: 4430
US Nat'l Entry Date:	October 6, 2005	
For:	SUBSTITUTED BENZOPYRANS AS SELECTIVE ESTROGEN RECEPTOR-BETA AGONISTS	
Docket No.:	X-16067	

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Venkatesh Krishnan, residing at 11161 Mirador Lane, Fishers, Indiana, declare that:

1. I hold the degree of doctor of philosophy in Biochemistry. I received my degree from Texas A&M University, College Station, Texas. I spent four years at Baylor College of Medicine, Houston, Texas for my postdoctoral fellowship.

2. I have been continuously employed since 1998 by Eli Lilly and Company in the following capacities: as a Sr. Research Advisor (October 2006 to present) Research Advisor (January, 2004 to September 2006), Principal Research Scientist (January, 2001-December, 2003) and Senior Scientist (February, 1998-December, 2000).

3. During my employment at Eli Lilly and Company, my research activities have included developing, optimizing and operation of in vitro, and in vivo (small animal) assays and supervising biologists who would carry out these assays. These assays provide preclinical

data on chemical compounds as potential pharmaceutical agents for the treatment of diseases or disorders in humans.

4. I have authored or co-authored 35 peer reviewed articles.

5. I have authored or co-authored 3 published or in press review articles on estrogen or estrogen receptor modulator interactions on human physiology.

6. I am a named co-inventor on 4 issued United States patents and additional pending U.S. patent applications.

7. Upon information and belief, I am a co-inventor of the subject matter described and claimed in U.S. Patent Application Serial No. 10/552,504, the application identified above.

8. Since February, 1998, my responsibilities have included being in charge of a laboratory in which among other assays Estrogen Receptor Binding Assays, Cell-Based Transcriptional Assays, Small Animal Model Prostate and Hormone Analysis Assays have been carried out and supervising biologists who carried out these assays on various chemical compounds. My responsibilities included recording and reporting and supervising biologists reporting to me in recording and reporting to other Eli Lilly and Company employees data generated on chemical compounds tested in those assays.

9. Upon information and belief, many of these chemical compounds are synthesized by Eli Lilly and Company chemists.

10. Upon information and belief, sample amounts of compounds sufficient for testing in biological assays are provided by Eli Lilly and Company chemists to scientists whose responsibilities include carrying out assays.

11. Upon information and belief, samples of some of the compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 in the application identified above were tested in one or more of the Estrogen Receptor (ER) Binding Assays, Cell Based Transcriptional Assays and Rat and Mouse Prostate and Hormone Analysis Assays. The assay protocols, data and results are described below.

12. ER Binding Assays.

These are the same assays described at page 103, line 16 through page 104, line 18 of the present patent application.

A cell free competition binding assay is run in a buffer containing 50mM N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulfonic acid] (HEPES), pH 7.5, 1.5mM EDTA, 150mM NaCl, 10% glycerol, 1mg/ml ovalbumin and 5mM dithiothreitol (DTT), using 0.025 µCi per well ^{3}H-Estradiol(New England Nuclear #NET517 at 118 Ci/mmol, 1 mCi/ml), 10 µg/well human ERalpha or human ERbeta receptor (PanVera-InVitrogen, Carlsbad, California). Test compounds are added at 10 different concentrations. Non-specific binding is

determined in the presence of 1μM of 17-B Estradiol (Sigma, St. Louis, MO). The binding reaction (140 μl) is incubated for 4 hours at room temperature, then 70 μl of cold dextran coated charcoal (DCC) buffer is added to each reaction (DCC buffer contains per 50 ml of assay buffer, 0.75g of charcoal (Sigma) and 0.25g of dextran (Pharmacia-Pfizer)). Plates are mixed 8 minutes on an orbital shaker at 4°C. Plates are then centrifuged at 3,000 rpm at 4°C for 10 minutes. An aliquot of 120μl of the mix is transferred to another 96-well, white flat bottom plate (Costar) and 175μl of Wallac Optiphase "Hisafe 3" scintillation fluid is added to each well. Plates are sealed and shaken vigorously on an orbital shaker. After an incubation of 2.5 hrs, the plates are read in a Wallac Microbeta counter. The data is used to calculate an IC50 and % Inhibition at 10μM. The K_d for ^{3}H-Estradiol is determined by saturation binding to ER alpha and ER beta receptors. The IC_{50} values for compounds are converted to K_i using Cheng-Prusoff equation.

13. Upon information and belief, the data for those exemplified compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 of the application identified above tested in the ER binding assays tested are provided in Exhibit 1 (attached).

14. Data in Exhibit 1 evidences a tested compound's binding affinity to each of the human ER alpha and human ER beta receptors and the individual compound's selectivity for binding to the human ER beta receptor compared to the human ER alpha receptor. Compounds that are selective for binding to the human ER beta receptor compared to the human ER alpha receptor bind to the human ER beta receptor with a lower Ki value compared to the Ki value for the human ER alpha receptor.

15. The data in Exhibit 1 generally evidences that the compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 have binding affinity and binding selectivity to ER beta over ER alpha.

16. PC-3 ERE Reporter Assay:

PC-3 human prostatic adenocarcinoma cell line (from metastatic site at bone, ATCC, Manassas, Virginia) was cultured in RPMI 1640 media without phenol red in the presence of 100 units penicillin, 100 g/ml streptomycin and 10% Heat inactivated Fetal Bovine Serum (FBS) (Gibco BRL, Rockville, MD). One day prior to the experiment 10 million cells were seeded onto RPMI 1640 media without phenol red in the presence of 10% charcoal stripped Heat inactivated FBS (Gibco BRL, Rockville, MD), 100 units penicillin and 100 g/ml streptomycin in a T150 tissue culture flask. Cells were transfected with 200μl Fugene 6 (Roche Biochemicals, Mannheim, Germany), 100μg plasmid containing 3x human ERE response element upstream of minimal thymidine kinase promoter driving luciferase gene, and 10μg of expression vector for full length human hERalpha or hERbeta receptor driven by viral cytomegalovirus (CMV) promoter (pCMVhER-alpha or pCMVhER-beta

expression plasmids). After 5 hours, cells were transferred to a 96-well tissue culture dish and the media was replaced with RPMI 1640 media without phenol red, in the presence of 10% charcoal stripped Heat inactivated FBS (Gibco BRL, Rockville, MD) without penicillin/streptomycin. Cells were treated with test compounds at different concentrations from 10μM and 10 point concentration dilution. Forty-eight hours later, cells were harvested and assayed for luciferase activity using a luciferin-CoA mixture. Chemiluminescent activity is measured as relative light units and EC50 for ER-alpha and ER-beta is calculated after fitting the data to a 4-parameter concentration response curve fit. The % efficacy was calculated vs diethylstilbestrol (DES) a synthetic non-selective estrogen agonist maximum response.

17. Upon information and belief, the data for those compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 tested in the PC-3 ERE reporter assays are provided in Exhibit 2 (attached).

18. Data in Exhibit 2 demonstrates activity and functional selectivity of those compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 tested in the PC-3 reporter assays for agonizing ER-beta over ER-alpha. Expression of a reporter gene (luciferase) by a test compound evidences agonist activity by that test compound while diminution of expression of that reporter gene (luciferase) by a test compound in the presence of or in comparison to a known agonist (DES) evidences antagonist activity by that test compound.

19. The date in Exhibit 2 generally evidences that the compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 tested in the PC-3 reporter assay demonstrate agonist activity and functional selectivity in eliciting a response by ER beta over ER alpha.

20. In vivo pharmacology in CD-1 mice

Upon information and belief, a mouse BPH assay is essentially performed as a modified version of the rat BPH assay described in (Eur. J. Endocrinol., 2004, Apr;150(4):591-603).

Compounds are evaluated for their effects upon the weight of the ventral prostate. Thirteen week old sexually mature CD-1, BALB/C male mice (Charles River Laboratories, Wilmington, Massachusetts) age matched and weight matched are single caged under alternate 12h dark and light cycles with water and powdered Rodent Laboratory Chow (Ralston-Purina, St. Louis, Missouri) supplied ad libitum. These investigations were conducted under practices outlines for the care and use of laboratory animals set forth by the U.S. National Institutes of Health and the American Association for Laboratory Animal Care. The animals are randomly assigned and treated with vehicle or compounds at various daily

doses, given orally (gavage) in a 1.25% Carboxymethylcellulose (CMC) + 0.125% Tween 20 in PBS, pH 6.8 formulation for 14 days. Each treatment arm had 8 or 10 animals. At the end of the study, final body weights are recorded, the animals are sacrificed using CO_2, followed by blood collection using cardiac puncture. The animals are then subjected to necropsy to collect intact ventral prostate lobes to measure organ wet weight changes between treatment groups. Ventral prostate weights (normalized to Body Weight) compared to vehicle control are analyzed using Dunnet's test (JMP, SAS Institute, Cary, North Carolina) expressed as significantly different ($p<0.05$) lower than vehicle treated controls using Fisher's least significant difference.

21. Upon information and belief, the results are shown as organ wet weights corresponding to each treatment group (Exhibit 3, attached).

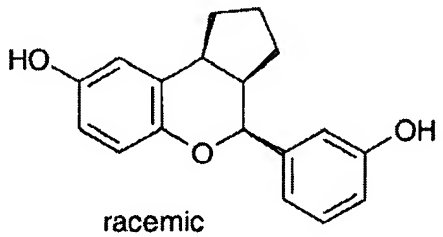
22. Benign prostatic hyperplasia (BPH) is characterized, in part, by an enlargement of the prostate gland. As the prostate gland enlarges, the weight of this gland will also increase. Compounds that reduce the weight of the prostate in small animal models, in vivo, demonstrate activity against BPH.

23. The data provided in Exhibit 3 evidences in vivo efficacy for treating BPH by a statistically significant lowering of ventral prostate weight by the administration of the compound of Examples 10-Enantiomer A and 12-Enantiomer A in a small animal model at the indicated doses.

24. Upon information and belief, I am a co-inventor of subject matter disclosed and claimed in U.S. Patent 6,630,508 B1 and U.S. Patent 7,217,734 B2.

25. Upon information and belief, the compound of Example 5 of U.S. Patent 6,630,508 B1 was synthesized by Eli Lilly and Company chemists, and sample amounts of that compound sufficient for testing in biological activity assays was provided to Eli Lilly and Company scientists whose responsibilities include carrying out biological activity assays.

26. Upon information and belief, the compound of Example 5 of U.S. Patent 6,630,508 B1 is (±)-2-(3-hydroxyphenyl)-6-hydroxy-cyclopentyl[c]3,4-dihydro-2H-1-benzopyran having the structure

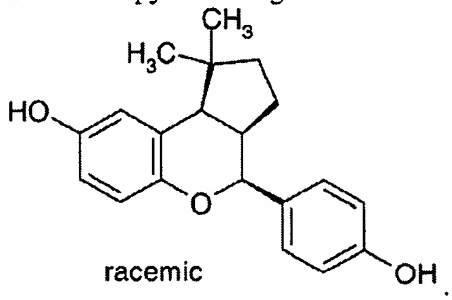


racemic

27. Upon information and belief, data obtained from the biological activity assay evaluation of the compound of Example 5 of U.S. Patent 6,630,508 B1 is provided in Exhibit 4 (attached).

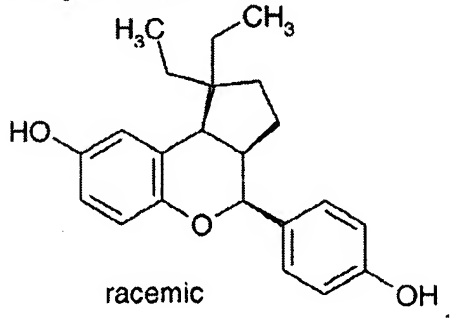
28. Upon information and belief, the compounds of Examples 10 and 11 of U.S. Patent 7,217,734 B2 were synthesized by Eli Lilly and Company chemists and sample amounts of those compounds sufficient for testing in biological activity assays were provided to Eli Lilly and Company scientists whose responsibilities include carrying out biological activity assays.

29. Upon information and belief, the compound of Example 10 of U.S. Patent 7,217,734 B2 is (+)-2-(4-hydroxyphenyl)-6-hydroxy-11,11-dimethyl-cyclopentyl[c]3,4-dihydro-2H-1-benzopyran having the structure



30. Upon information and belief, data obtained from the biological activity assay evaluations of the compound of Example 10 of U.S. Patent 7,217,734 B2 is provided in Exhibit 4 (attached).

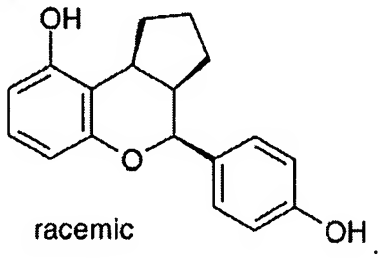
31. Upon information and belief, the compound of Example 11 of U.S. Patent 7,217,734 B2 is (+)-2-(4-hydroxyphenyl)-6-hydroxy-11,11-diethyl-cyclopentyl[c]3,4-dihydro-2H-1-benzopyran having the structure



32. Upon information and belief, data obtained from biological activity assay evaluations of the compound of Example 11 of U.S. Patent 7,217,734 B2 is provided in Exhibit 4 (attached).

33. Upon information and belief, the compound of Example 5 of U.S. Patent 7,217,734 B2 was synthesized by Eli Lilly and Company chemists, and sample amounts of that compound sufficient for testing in biological activity assays was provided to Eli Lilly and Company scientists whose responsibilities include carrying out biological activity assays.

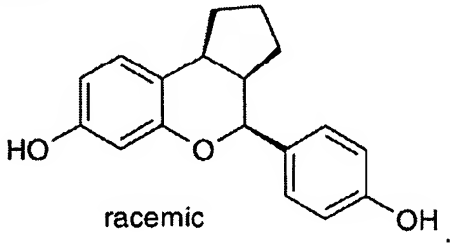
34. Upon information and belief, the compound of Example 5 of U.S. Patent 7,217,734 B2 is (±)-2-(4-hydroxyphenyl)-5-hydroxy-cyclopentyl[c]3,4-dihydro-2H-1-benzopyran having the structure



35. Upon information and belief, data obtained from the biological activity assay evaluation of the compound of Example 5 of U.S. Patent 7,217,734 B2 is provided in Exhibit 4 (attached).

36. Upon information and belief, the compound of Example 6 of U.S. Patent 7,217,734 B2 was synthesized by Eli Lilly and Company chemists and sample amounts of that compound sufficient for testing in biological activity assays was provided to Eli Lilly and Company scientists whose responsibilities include carrying out biological activity assays.

37. Upon information and belief, the compound of Example 6 of U.S. Patent 7,217,734 B2 is (±)-2-(4-hydroxyphenyl)-7-hydroxy-cyclopentyl[c]3,4-dihydro-2H-1-benzopyran having the structure



38. Upon information and belief, data obtained from the biological activity assay evaluations of the compound of Example 6 of U.S. Patent 7,217,734 B2 is provided in Exhibit 4 (attached).

39. Based upon the data in Exhibit 4, the compound of Example 5 in U.S. Patent 6,630,508 B1 evidences Binding Selectivity for ER beta over ER alpha of approximately 3.5.

40. Based upon the data in Exhibit 4, the compound of Example 10 in U.S. Patent 7,217,734 B2 evidences Binding Selectivity for ER beta over ER alpha of approximately 2.

41. Based upon the data in Exhibit 4, the compound of Example 11 in U.S. Patent 7,217,734 B2 evidences Binding Selectivity for ER beta over ER alpha of approximately 2.

42. Based upon the data in Exhibit 4, the compound of Example 5 in U.S. Patent 7,217,734 B2 evidences Binding Selectivity for ER beta over ER alpha of approximately 0.5.

43. Based upon the data in Exhibit 4, the compound of Example 6 in U.S. Patent 7,217,734 B2 evidences Binding Selectivity for ER beta over ER alpha of approximately 2.3.

44. Based upon the data in Exhibit 1, for each of the compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 for which data exists of U.S. Patent Application Serial No. 10/552,504, the application identified above, the compounds identified as and/or in Examples 8-13, 15-18, 24, 28 and 32 generally evidence improved Binding Selectivity over the data for each of the comparative compounds provided in Exhibit 4.

45. Upon information and belief of the structures of the compound of Example 5 of U.S. Patent 6,630,508 B1, compounds of Examples 10, 11, 5 and 6 of U.S. Patent 7,217,734 B2 and the structures for each of the compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32 of U.S. Patent Application Serial No. 10/552,504, the application identified above, and the data in Exhibits 1 and 4, the addition of a substituent as identified for Examples 8-13, 15-18, 24-28 and 32 at the apex carbon atom of the cyclopentyl fused ring portion of the molecule generally affords an unexpected improvement in Binding Selectivity over the Binding Selectivity for each of Example 5 of U.S. Patent 6,630,508 B1 and Examples 10, 11, 5 and 6 of U.S. Patent 7,217,734 B2.

46. Upon information and belief, the data reported in and as Exhibits to this Declaration are reported as arithmetic values, unless otherwise specified.

47. Upon information and belief, data in U.S. Patent 6,630,508 B1, U.S. Patent 7,217,734 B2, and my previous Declaration submitted in U.S. Patent 7,217,734 B2 are all provided as or based upon arithmetic values.

48. Upon information and belief, additional published and unpublished data from various assays exists including in vitro, in vivo (non-human animal) models, or

both, for one or more of the compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32.

49. Upon information and belief, the data presented in this Declaration is not contrary to the totality of data identified above in Paragraph 48.

50. Upon information and belief, any differences between data contained in and with this Declaration and published or unpublished reports of data on the compounds identified as and/or in Examples 8-13, 15-18, 24-28 and 32, included in the patent application identified above, Example 5 of U.S. Patent 6,630,508 B1, and Examples 10, 11, 5 and 6 of U.S. Patent 7,217,734 B2 are within experimental error and result from multiple assays or multiple assay runs and do not significantly affect the interpretation of data or conclusions made in this Declaration.

51. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Venkatesh Krishnan

Venkatesh Krishnan

APRIL 01, 2008

Date

	Recombinant Full Length ER-alpha Competition Binding		Recombinant Full Length ER-beta Competition Binding		Binding Selectivity
Example No.	avg Ki (nM) ± Standard Deviation	Number of assays	avg Ki (nM) ± Standard Deviation	Number of assays	ERa (Ki nM)/ ERb (Ki nM)
8-Racemic	805 (2 assays >1100 nM)	3	11.2 ± 3.56	2	71.9
8-Enantiomer A	698 ± 169	4	6.92 ± 0.62	4	101
8-Enantiomer B	>1100	2	60.5 ± 0.92	2	>18
9-Racemic	>1100	2	450 ± 102	2	>2.4
10-Racemic	23.9	1	0.72	1	33.2
10-Enantiomer A	8.33 ± 2.73	14	0.45 ± 0.26	14	18.5
10-Enantiomer B	68.3 ± 19.9	2	2.62 ± 0.45	2	26.1
11-Racemic	32.9	1	1.33	1	24.7
11-Enantiomer A	12.4 ± 4.92	3	0.70 ± 0.45	3	17.7
11-Enantiomer B	261	1	6.11	1	42.7
12-Racemic	28.1	1	1.32	1	21.3
12-Enantiomer A	7.17 ± 0.75	5	0.41 ± 0.069	5	17.5
12-Enantiomer B	113	1	6.36	1	17.8
13-Racemic	>1100	1	405	1	>2.7
15-Racemic	>1100	1	98.9	1	>11
15-Enantiomer A	404	1	60.8	1	6.6
15-Enantiomer B	686	1	79.5	1	8.6

16-Racemic	>1100	1	137	1	>8
16-Enantomer A	>1100	1	62.3	1	>17
16-Enantiomer B	>1100	1	213	1	>5
17-Racemic	15.7	1	1.40	1	11.2
17-Enantiomer A	16.1 ± 5.94	2	0.68 ± 0.22	2	23.7
17-Enantiomer B	42.7	1	4.03	1	10.6
18-Racemic	250	1	8.17	1	30.6
24-Racemic	377	1	63.9	1	5.90
25-Racemic	54.2	1	4.92	1	11.0
25-Enantiomer A	11.8	1	2.32	1	5.1
25-Enantiomer B	714	1	11.1	1	64.3
26-Racemic	7.29	1	0.35	1	20.8
26-Enantiomer A	4.51 ± 1.59	2	0.23 ± 0.092	2	19.6
26-Enantiomer B	44.4	1	2.01	1	22.1
27-Racemic	5.28	1	0.46	1	11.5
28-Racemic	35.9	1	1.59	1	22.6
32-Racemic	50.1	1	6.12	1	8.2

	ERE ER-alpha Cotransfection assay PC3 cells		ERE ER-beta Cotransfection assay PC3 cell		Functional Selectivity
Example No.	Avg EC50 (nM) ± Standard Deviation	Number of assays	Avg EC50 (nM) ± Standard Deviation	Number of assays	ER-alpha (EC50 nM)/ ER-beta (EC50 nM)
8-Racemic	198 ± 30.3	4	22.9 ± 4.37	4	33
8-Enantiomer A	187 ± 80.4	4	12.5 ± 5.19	4	48
8-Enantiomer B	789 (for 2 assays, EC50 not calculated)	3	96.9 ± 18.7	3	31
9-Racemic	>10000 (for 2 assays, EC50 not calculated)	3	433 ± 147	3	
10-Racemic	91.2 ± 25.2	2	0.96 ± 0.070	2	126
10-Enantiomer A	47.6 ± 26.7	31	0.85 ± 0.48	32	71
10-Enantiomer B	159 ± 119	18	6.70 ± 3.10	18	52
11-Racemic	88.3 ± 36.3	2	2.06 ± 0.099	2	45
11-Enantiomer A	39.6 ± 16.3	9	1.26 ± 0.611	9	37
11-Enantiomer B	525 ± 122	2	33.4 ± 2.90	2	19
12-Racemic	82.9 ± 17.9	2	2.46 ± 0.403	2	45
12-Enantiomer A	65.6 ± 31.6	9	0.943 ± 0.389	8	69.5
12-Enantiomer B	1267 ± 401	2	70.5 ± 0.141	2	18
13-Racemic	>10000	1	408	1	>24
15-Racemic	1142 ± 1001	2	58.6 ± 11.1	2	19.5

15-Enantiomer A	>10000, <10000	2	54.7 ± 3.89	2	
15-Enantiomer B	4940	1	165	1	30
16-Racemic	>10000	2	347 ± 112	2	>28
16-Enantiomer A	>10000, <10000	2	194 ± 44.5	2	
16-Enantiomer B	>10000	2	1075 ± 601	2	>9
17-Racemic	25.4	1	1.39	1	18.3
17-Enantiomer A	27.4 ± 15.2	4	1.14 ± 0.449	4	8
17-Enantiomer B	60.1	1	3.28	1	18.3
18-Racemic	443	1	6.89	1	64.3
24-Racemic	>10000	2	65.5 ± 32.5	2	>152
25-Racemic	161 ± 17.0	2	11.2 ± 0.636	2	14.4
25-Enantiomer A	67.8	1	2.10	1	32.3
25-Enantiomer B	>10000	1	14.8	1	>675
26-Racemic	Not tested	----	Not tested	----	----
26-Enantiomer A	22.2 ± 3.11	5	0.824 ± 0.129	5	27
26-Enantiomer B	145 ± 14	3	10.9 ± 2.32	3	13.4
27-Racemic	37.8 ± 13.1	4	1.83 ± 0.152	4	21
28-Racemic	Not tested	----	Not tested	----	----
32	<10000	1	30.1	1	>330

Prostate weights in CD-1 mice after treatment

Treatment	Prostate Weight (mg) Mean ± Standard Deviation	Body weight (g) Mean ± Standard Deviation	Prostate Weight (mg)/ Body Weight (g)
Example 10-Enantiomer A 0.01 mg/kg	17.84 ± 2.73	38.19 ± 2.18	0.47
Example 10-Enantiomer A 0.1 mg/kg	15.68 ± 1.20	39.60 ± 2.78	0.40*
Example 10-Enantiomer A 1.0 mg/kg	16.56 ± 1.89	38.63 ± 1.92	0.43*
Example 10-Enantiomer A 10 mg/kg	14.93 ± 3.67	40.76 ± 3.59	0.37*
Vehicle Control	20.13 ± 2.70	39.30 ± 2.74	0.51
Castration (n = 5 animals)	4.10 ± 1.18	40.12 ± 3.54	0.10

n = 8 animals per group, * $p<0.05$ significantly lower than Vehicle Control

Treatment	Prostate Weight (mg) Mean ± Standard Deviation	Body weight (g) Mean ± Standard Deviation	Prostate Weight (mg)/ Body Weight (g)
Example 12-Enantiomer A 0.01 mg/kg	15.63 ± 6.65	39.77 ± 1.77	0.39*
Example 12-Enantiomer A 0.1 mg/kg	13.98 ± 3.02	38.29 ± 1.21	0.36*
Example 12-Enantiomer A 1.0 mg/kg	15.54 ± 4.31	39.04 ± 2.30	0.40*
Example 12-Enantiomer A 10 mg/kg	13.65 ± 3.09	38.11 ± 0.74	0.36*
Vehicle Control	23.40 ± 4.43	39.36 ± 1.16	0.59
Castration (n = 5 animals)	4.02 ± 1.22	42.02 ± 2.31	0.096

n = 10 animals per group, * $p<0.05$ significantly lower than Vehicle Control

Treatment	Prostate Weight (mg) Mean ± Standard Deviation	Body weight (g) Mean ± Standard Deviation	Prostate Weight (mg)/ Body Weight (g)
Example 10-Enantiomer A 0.01 mg/kg	16.90 ± 3.00	34.69 ± 1.35	0.49
Example 10-Enantiomer A 0.05 mg/kg	12.58 ± 2.22	33.61 ± 1.41	0.37*
Example 10-Enantiomer A 0.1 mg/kg	12.03 ± 1.21	35.55 ± 1.82	0.34*
Example 10-Enantiomer A 0.1 mg/kg# (n = 4 animals)	14.00 ± 3.42	35.34 ± 1.22	0.40*
Vehicle Control	17.41 ± 2.06	33.83 ± 1.45	0.51
Castration (n = 5 animals)	1.98 ± 0.74	33.49 ± 1.76	0.059*

n = 8 animals per group, * $p<0.05$ significantly lower than Vehicle Control

#this group received one dose of 0.1 mg/kg on day 7 of the experiment

Treatment	Prostate Weight (mg) Mean ± Standard Deviation	Body weight (g) Mean ± Standard Deviation	Prostate Weight (mg)/ Body Weight (g)
Example 12-Enantiomer A 0.001 mg/kg	17.20 ± 2.38	35.42 ± 1.82	0.49
Example 12-Enantiomer A 0.01 mg/kg	12.62 ± 2.59	36.84 ± 1.83	0.34*
Example 12-Enantiomer A 0.1 mg/kg	12.82 ± 2.33	34.97 ± 1.69	0.37*
Example 12-Enantiomer A 1.0 mg/kg	11.95 ± 2.39	35.55 ± 1.81	0.34*
Vehicle Control	18.60 ± 3.78	35.85 ± 1.80	0.52
Castration (n = 5 animals)	1.38 ± 1.10	35.00 ± 1.25	0.039*

n = 8 animals for vehicle control; n = 10 animals per treatment group, * $p<0.05$ significantly lower than Vehicle Control

Comparative Compound	Recombinant Full Length ER-alpha Competition Binding avg Ki(nM) ± Standard Deviation	Recombinant Full Length ER-beta Competition Binding avg Ki (nM) ± Standard Deviation	Number of Assays	Binding Selectivity ERa (Ki nM)/ERb (Ki nM)	ERE ER-alpha Cotransfection assay PC3 cells EC50 (nM)	ERE ER-beta Cotransfection assay PC3 cells EC50 (nM)	Number of Assays
Compound of Example 5 from U.S. Patent 6,630,508 B1	68.8 ± 30.8	19.5 ± 7.37	4	3.5	N.T.[a]	N.T.	
Compound of Example 10 from U.S. Patent 7,217,734 B2	13.90 ± 0.42	6.26 ± 0.81	2	2	60.51	25.94	1
Compound of Example 11 from U.S. Patent 7,217,734 B2	46.75 ± 9.26	28.81 ± 38.03	2	2	N.T.	N.T.	
Compound of Example 5 from U.S. Patent 7,217,734 B2	0.348 ± 0.043	0.668 ± 0.077	4	0.52	N.T.	N.T.	
Compound of Example 6 from U.S. Patent 7,217,734 B2	43.30 ± 17.0	18.9 ± 17.0	5	2.3	N.T.	N.T.	

[a] NT is not tested